NEUROPHARMACOLOGICAL STUDIES OF CAPSAICIN FOR THE MANAGEMENT OF COMORBID BRAIN DISORDERS

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EXECUTIVE SUMMARY

Background: Neurological disorders are a combination of pathological conditions that precede slow and progressive deformations and induce loss of neurons and synapses in certain areas of the nervous system and associated functions. Even though the basic molecular mechanism of neurological disorders remains unclear, a combination of several factors, including endogenous, genetic, and environmental elements related to ageing, contributes to the generation of the disease. Excessive generation of Mitochondrial ROS and alteration in mitochondrial redox homeostasis are involved in several neurodegenerative diseases. Overstimulation of glutamate receptors is responsible for the death of neuronal cells (glutamate excitotoxicity), which contributes to one major mechanism in the degeneration of neurons. Chronic inflammation in neurons is also the key factor in most neurodegenerative disorders. As the phytochemicals exert a protective nature in different neurological diseases, there is a huge level of exploration to determine their potency to manage these diseases. Capsaicin is one of the major phenolic components present in red chilli and is responsible for its aromatic and spicy flavour. Capsaicin has an agonistic effect on TRPV1 channels and is also reported to have a neuroprotective role. Capsaicin is effective against oxidative damage, the Streptozotocin-induced Alzheimer's model, and 6-OHDA-induced Parkinson's disease. It also possesses anticonvulsant and neuroprotective effects in pentylenetetrazol-induced seizures and global cerebral ischemia in the Mongolian gerbil. Since TRPV1 plays a dominant role in the protective action of capsaicin, it is essential to understand its significance with relevance to the different pathways that lead to neuronal cell death. Therefore, new drugs targeting the TRPV1 (capsaicin receptor) open a promising horizon of pharmacological advances in the years to come for managing neurological disorders.

Aim and objective: The study objectives are to explore the neuroprotective effect of capsaicin in glutamate and Lipopolysaccharides (LPS) induced neurotoxicity in Neuro2a (N2a) cells as well as the potential role in treatment and management of cognitive impairment, mitochondrial deficits, neuroinflammation, and oxidative insult through MPTP (1-methyl-4-phenyl-1, 2, 3, 6- tetrahydropyridine), scopolamine and 3-nitropropanoic acid-induced model of PD, AD, and HD in Swiss albino mice.

Material and Methods: Cell viability was determined by MTT and LDH assay in LPS and glutamate-induced N2a cell line ROS levels, blood cytokines, mitochondrial

membrane potential for mitochondrial impairment, and oxidative stress parameters like SOD and LPO were also performed in the N2a cells challenged to glutamate (10 mM) and LPS (1 μg/ml).

In the mice model of PD, after the systemic administration of four doses of MPTP (23 mg/kg *i.p.*), every other day from the first day (day 1 to day 7), capsaicin (5, 10, and 20 mg/kg) was given orally for seven consecutive days in mice. Behavioural test parameters (resting tremor score, muscle coordination, locomotor counts, hole board test, tail suspension test and hot plate test) were observed and biochemical analysis (total antioxidant capacity, lipid peroxidation, superoxide dismutase, reduced glutathione, catalase, nitrite, and cytokines level) in plasma, mitochondrial complex activities (I, II, III, and IV), and mitochondrial permeability transition in isolated mitochondrial pellets were estimated.

In the scopolamine-induced AD mice model, various doses of capsaicin (5, 10, and 20 mg/kg) were given orally to mice daily for 7 consecutive days after the administration of scopolamine (1 mg/kg, *i.p.*). Various behavioural tests (motor coordination, locomotor counts, hole board test, forced swim test, learning memory and anxiety by light and dark model) and biochemical assay (Pro-inflammatory cytokines, catalase, lipid peroxidation, nitrite, reduced glutathione, and superoxide dismutase), mitochondrial complex enzyme (I, II, III, and IV) activities, and mitochondrial permeability transition were evaluated in the distinct regions of the brain.

In 3-Nitropropionic acid-induced Huntington model in mice, 3-NP (25 mg/kg) administered mice were treated with capsaicin (5, 10, and 20 mg/kg) through the *per-oral (p.o.)* route for 7 consecutive days. Physiological and behavioural studies were performed in drug-treated mice. After behavioural studies, biochemical and histochemical examinations were performed for cytokines levels, various oxidative stress parameters, and mitochondrial enzyme complex activities with mitochondrial permeability.

Results: In the cell line study, CAP at various doses (5, 10, 25, and 50 μM) altered the changes induced by glutamate and LPS in cell morphology and restored the oxidative, cytokines levels and loss of mitochondrial membrane integrity, but CAP (100 μM) showed similar toxicity as in glutamate and LPS control group which was also evident in cytotoxicity (MTT and LDH) assay. MPTP-administered mice demonstrated impaired behaviour in various behavioural tests, elevated resting tremors and immobility period,

reduced locomotor activity, exploratory behaviour, latency fall of time by affecting its grip strength increased oxidative stress and cytokine level, and impaired mitochondrial functions compared to the normal control mice. Capsaicin treatment restored the behavioural parameters studied and modulated antioxidant activities, nitric oxide levels, cytokines level, mitochondrial complexes activities, and mitochondrial permeability. Moreover, IL-1β level restored toward normal at 20 mg/kg of capsaicin *p.o.* only, whereas, for IL-6 and TNF-α, the effect was shown by capsaicin 10 and 20 mg/kg *p.o*. Preservation of neuronal morphology by capsaicin was also confirmed by histological studies.

Scopolamine-treated mice showed a considerable reduction in the entries and duration in the light zone in the light and dark model as well as in open arms of the elevated plus maze respectively. Interestingly, capsaicin at different doses reversed the anxiety, depressive-like behaviours, and learning and memory impairment effects of scopolamine. Scopolamine-administered mice demonstrated substantially increased cytokines levels, impaired mitochondrial enzyme complex activities, and increased oxidative damage compared to the normal control group. Capsaicin treatment reinstated the reduced lipid peroxidation, nitric oxide, catalase, superoxide dismutase, reduced glutathione activity, decreasing cytokines and restoring mitochondrial complex enzyme activities (I, II, III, and IV) as well as mitochondrial permeability. Moreover, the IL-1β level was restored at a dose of capsaicin (10 and 20 mg/kg) *p.o.* only. Capsaicin reduced the scopolamine-induced acetylcholinesterase activity, thereby raising the acetylcholine concentration in the hippocampal tissues of mice. Preservation of neuronal cell morphology was also confirmed by capsaicin in histological studies. From the above experimental results, 10 mg/kg of capsaicin, *p.o.* for seven repeated days was found to be the most effective dose.

All tested doses of capsaicin upturned the cognitive impairment and motor in-coordination effects induced by 3-NP. 3-NP-injected mice demonstrated substantially increased cytokine levels, defective mitochondrial complex activity, and augmented oxidative insult. However, capsaicin at different doses reduced oxidative damage and cytokines levels and improved mitochondrial complex activity along with mitochondrial permeability. Furthermore, capsaicin (10 and 20 mg/kg, *p.o.*) improved the TNF-α level. Histological studies revealed the 3-NP injected mice (striatum region) showed a severe distortion in striatum neurons and, the presence of nuclear pyknosis in a region which led

to neuronal cell death. However, after the treatment of CAP at concentrations (5, 10, and 20 mg/kg) no nuclear pyknosis was found and restoration of distorted striatal neurons was also observed.

Conclusions: These findings through (*in-vitro and in-vivo)* studies suggested the neuroprotective activity of capsaicin which can be considered a novel treatment as a neuroprotectant for the treatment and management of neurodegenerative disorders like PD, AD, and HD by restoring the antioxidant enzyme activity, cytokine levels, and mitochondrial functions.